PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
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in its capacity as elected Office

Date of mailing (day/month/year) 20 January 1998 (20.01.98)	
International application No. PCT/AU97/00436	**Applicant's or agent's file reference**
International filing date (day/month/year) 09 July 1997 (09.07.97)	**Priority date** (day/month/year) 09 July 1996 (09.07.96)
Applicant MENDELSOHN, Frederick, A., O. et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   08 January 1998 (08.01.98)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference VS:LM:FP4736	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. **PCT/AU 97/00436**	International filing date 9 July 1997	Priority Date 9 July 1996

International Patent Classification (IPC) or national classification and IPC

Int. Cl.[6] C07K 7/14, 16/28; A61K 31/70, 38/08; C12N 15/12

Applicant
(1) HOWARD FLOREY INSTITUTE OF EXPERIMENTAL PHYSIOLOGY AND MEDICINE
(2) MENDELSOHN, Frederick A O et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of **four** sheets, including this cover sheet.

[X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 7 sheet(s).

3. This report contains indications relating to the following items:

I	[X]	Basis of the report
II	[ ]	Priority
III	[X]	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[ ]	Certain defects in the international application
VIII	[ ]	Certain observations on the international application

Date of submission of the demand 8 January 1998	Date of completion of the report 30 June 1998
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. (02) 6285 3929	Authorized Officer **J.H. CHAN** Telephone No. (02) 6283 2340

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/[redacted]97/00436

I. Basis of the report

1. This report has been drawn on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

☐ the international application as originally filed.

☒ the description, pages **1-2, 6-44,** as originally filed,
pages , filed with the demand,
pages **3-5a,** filed with the letter of **7 May 1998 ,**
pages , filed with the letter of .

☒ the claims, Nos. , as originally filed,
Nos. , as amended under Article 19,
Nos. , filed with the demand,
Nos. **1-17 ,** filed with the letter of **7 May 1998 ,**
Nos. , filed with the letter of .

☒ the drawings, sheets/fig **1/15-15/15 ,** as originally filed,
sheets/fig , filed with the demand,
sheets/fig , filed with the letter of ,
sheets/fig , filed with the letter of .

2. The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application,

☒ claims Nos.: **11-17**

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claim Nos. **11-17**

International application No.
PCT/AU 97/00436

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-10	YES
	Claims		NO
Inventive step (IS)	Claims	1-10	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-10	YES
	Claims		NO

2. Citations and explanations

D1 Journal of Neurochemistry (June 1997), volume 68, no: 6, pages 2530-7, I Moeller et al, "the Globin fragment LVV-hemorphin-7 is an endogenous ligand for the AT receptor in the brain"

D2 Neuropeptides (1995), volume 28, pages 243-250, I Garreau et al; VV-hemorphin-7 and LVV-hemorphin-7 released during in vitro peptic haemoglobin hydrolysis are morphinominetic peptides"

D3 Biochemical and Biophysical Research Communications (1992), volume 189, no: 1, J Piot et al; "Isolation and characterisation of two opioid peptides from a bovine haemoglobin peptic hydrolysate"

D4 Biochemical and Biophysical Research Communications (1992), volume 184, no: 2, E Giamsta et al; pages 1060-1066 "Isolation of a haemoglobin-derived opioid peptide from cerebrospinal fluid of patients with cerebrovascular bleedings"

D5 Biochimica et Biophysica Acta (1980) volume 625, pages 266-273, R Chang et al; "Isolation and structure of several peptides from porcine hypothalami"

D6 Neurobiology (1996) volume 4, no: 3, pages 279-280, J Szikrat & A Borsodi; "Receptor binding properties of a hemorphin analogue in rat brain membrane preparations"

D7 Biochemical and Biophysical Research Communications (1994), volume 202, no: 1, pages 410-415, A Karelin et al; "Isolation of endogenous hemorphin-related haemoglobin fragments from bovine brain"

D8 Nucleic Acids Research (1989), volume 17, no: 21, page 8870, C Woo et al; "cDNA sequences of two β-globin genes in Sprague-Dawley rat" (embl accession numbers M17084 and X16417)

New Citations

D9 EMBL accession numbers M94918, X05080, X67613, S71213 and X15009. Swiss protein accession numbers P02091 and P33584.

Whilst claims 1-10 are to the subject matter - Method for treatment of the human or animal body by surgery or therapy - which are excluded according to Rule 67.1 of the PCT, the novelty, inventive step and industrial applicability of these claims have been examined because the subject matter claimed does not contravene the Australian Patent Law.

Document D1 discloses LVV-hemorphin 7 behaves as a high-affinity ligand for angiotensin IV receptor. However document D1 is published after the priority date of the application, and unless the priority date of the application is challenged, document D1 cannot form part of the prior art as defined in Rule 64.1 of the PCT.

None of the documents D2-D9 discloses the specific pharmacological activity of LVV hemorphin 7 (its high affinity to angiotensin IV receptor) leading to a possible therapeutic use. For these reasons the invention as defined in claims 1-10 is novel and inventive.

Claims 1-10 has industrial applicability according to the Australian Patent Law.

been associated with the regulation of neuronal development. Acetylcholine inhibits neurite outgrowth from embryonic chicken ciliary ganglion cells and sympathetic neurons (Pugh and Berg, 1994; Small *et al*, 1995), and rat hippocampal neurons (Muttson, 1988). Conversely, vasoactive intestinal peptide stimulates superior cervical ganglion branching (Pincus *et al*, 1990) and somatostatin increases neuronal sprouting from *Helisoma* buccal ganglion neurons (Bulloch, 1987).

We have now surprisingly found that the peptide LVV-haemorphin-7, derived from β-globin, acts as an agonist at the AT_4 receptor, and is the endogenous ligand for the AT_4 receptors in the brain. We have characterised its pharmacological activity. This enables us to design novel agonists and antagonists of Ang IV action.

Summary of the Invention

According to a first aspect, the invention provides a method of modulating motor neuron activity, cholinergic neuron activity, or neuronal development, comprising the step of administering an effective amount of a neuroactive peptide having at least one of the biological activities of angiotensin IV as herein defined, comprising the amino acid sequence:

Leu-Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe, (SEQ ID NO:1)

or a biologically-active analogue or fragment of said peptide to a mammal in need of such treatment. This aspect of the invention specifically includes the use of decapeptide sequence referred to above in the method of the invention which relies on a previously unknown and unsuspected activity of the decapeptide.

It will be clearly understood that the sequence of the invention may be modified by conservative amino acid substitutions, insertions, deletions or extensions, provided that the biological activity is retained. Such variants may, for example, include sequences comprising D-amino acids, non-naturally occurring amino acids, and/or

amino acid analogues. Thus the analogue may be a peptidomimetic compound.

Preferably the mammal is a human.

The Ang IV agonist and antagonist compounds according to the invention are useful in the treatment of a variety of conditions, including but not limited to:

- Dementia, including Alzheimer's disease
- Other neurodegenerative disorders involving cholinergic pathways, motor pathways, or sensory pathways, such as motor neurone disease
- sensory and motor peripheral neuropathies
- brain or spinal cord injury due to trauma, hypoxia or vascular disease.

In a second aspect, the invention provides a non-peptide analogue of the peptide of the invention. This non-peptide analogue is to be understood to encompass modifications or substitutions of the peptide structure which are designed to improve the bioavailability, metabolic stability, half-life in the body, or to modify the biological activity, of the compound of the invention. Such non-peptide analogues are known in the art, for example compounds in which the peptide backbone is replaced by a non-peptide chain, and are often referred to as peptidomimetic compounds. Alternatively, in one or more of the peptide linkages the order of the nitrogen and carbon atoms can be reversed to form a pseudo peptide bond. One or more of the amino acid side-chains may be replaced by an analogous structure of greater stability. Many other such variations will occur to the person skilled in the art. The only requirement is that the overall 3-dimensional structure is sufficiently preserved that ability to bind to the AT_4 receptor at suitable affinity is retained. Using modern methods of peptide synthesis and combinatorial chemistry, it is possible to synthesize and test very large numbers of analogues within a short space of time, and such synthesis and screening is routinely carried out by pharmaceutical companies.

Considerable information is available regarding the structural features of Ang IV peptides which are necessary for high affinity, and these results may be used as guidelines for modification of the peptides of the invention. See for example Wright *et al*, 1995.

The person skilled in the art will appreciate that by modifying the sequence or by constructing a non-peptide analogue the activity of the compound of the invention can be very considerably modified. Not only can improvement in activity be obtained, it is also possible to obtain compounds which bind to the AT_4 receptor in such a way that Ang IV activity is inhibited. Such inhibitory compounds can have the ability to antagonize the activity of Ang IV. The person skilled in the art will readily be able to synthesize modified peptides and peptide analogues and to test whether they have activity as Ang IV agonists or antagonists, using methods well known in the art.

According to a third aspect, the invention provides a method of screening for putative agonists or antagonists of the effect of LVV-haemorphin-7 on neuronal activity, comprising the step of testing the ability of the compound to stimulate or inhibit the effect of LVV-haemorphin-7 on a biological activity selected from the group consisting of modifying learning, modifying behaviour, vasoactive effects, dilation of cerebral arteries, increase in renal blood flow, increase in stereotypy behaviour, facilitating memory retrieval, neurite modelling and alleviation of the effects of spinal cord injury.

Thus according to a fourth aspect, the invention also provides compounds which are able to act as agonists or antagonists of the neuroactive peptides of the invention.

Detailed Description of the Invention

The invention will be now described in detail by way of reference only to the following non-limiting

examples, and to the figures, in which

Figure 1 shows competition curves derived from prefrontal cortical sections incubated with [^{125}I]Ang IV in the presence of increasing concentrations of the following unlabelled ligands: ▲ Ang IV, □ Ang II, ■ Ang III, Δ Ang II(1-7), ● losartan and ○ PD 123319. Values are the mean of four sections, each from two animals. B/Bo x 100 expressed as a percentage available receptors occupied;

Figure 2 shows the results of competition binding studies showing the inhibition of [^{125}I]Ang IV binding to

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CLAIMS

1. A method of modulating neuronal activity, comprising the step of administering an effective amount of a neuroactive peptide having at least one of the biological activities of angiotensin IV as herein defined, comprising the amino acid sequence: Leu-Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe, (SEQ ID NO:1), or a biologically-active analogue or fragment of said peptide, to a mammal in need of such treatment.

2. A method of modulating neuronal activity, comprising the step of administering a biologically-active non-peptide analogue of the neuronal peptide according to claim 1 to a mammal in need of such treatment.

3. A method according to claim 2, in which the biologically-active analogue is a peptidomimetic compound.

4. A method according to any one of claims 1 to 3, in which the biological activity is selected from the group consisting of modifying learning, modifying behaviour, vasoactive effects, dilation of cerebral arteries, increase in renal blood flow, increase in stereotypy behaviour, facilitating memory retrieval, neurite modelling and alleviation of the effects of spinal cord injury.

5. A method according to any one of claims 1 to 4, wherein said neuronal activity is selected from the group consisting of motor neuron activity, cholinergic neuron activity and neuronal development.

6. A method of treating a condition selected from the group consisting of dementia; Alzheimer's disease; neuro-degenerative disorders involving one or more of cholinergic pathways, motor pathways, or sensory pathways; motor neuron disease; sensory peripheral neuropathies; motor peripheral neuropathies; brain injury; and spinal cord injury resulting from one or more trauma, hypoxia, and vascular disease, comprising the step of administering an effective amount of a neuroactive peptide having at least one of the biological activities of angiotensin IV as

herein defined, comprising the amino acid sequence: Leu-Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe, (SEQ ID NO:1), or a biologically-active analogue or fragment of said peptide, to a mammal in need of such treatment.

7. A method according to claim 6, comprising the step of administering a biologically-active non-peptide analogue of the neuroactive peptide of claim 6 to a subject in need of such treatment.

8. A method according to claim 7, in which the biologically-active analogue is a peptidomimetic compound.

9. A method according to any one of claims 6 to 8, in which the biological activity is selected from the group consisting of modifying learning, modifying behaviour, vasoactive effects, dilation of cerebral arteries, increase in renal blood flow, increase in stereotypy behaviour, facilitating memory retrieval, neurite modelling and alleviation of the effects of spinal cord injury.

10. A method according to any one of claims 1 to 9, in which the mammal is a human.

11. A method of screening for putative agonists or antagonists of the effect of LVV-haemorphin-7 on neuronal activity, comprising the step of testing the ability of the compound to stimulate or inhibit the effect of LVV-haemorphin-7 on a biological activity selected from the group consisting of modifying learning, modifying behaviour, vasoactive effects, dilation of cerebral arteries, increase in renal blood flow, increase in stereotypy behaviour, facilitating memory retrieval, neurite modelling and alleviation of the effects of spinal cord injury.

12. An antagonist of LVV-haemorphin-7, identified by the method of claim 11.

13. An agonist of LVV-haemorphin-7, identified by the method of claim 11.

14. A method of modulating neuronal activity, comprising the step of administering an effective amount of an antagonist according to claim 11 to a mammal in need of

such treatment.

15. A method of modulating neuronal activity, comprising the step of administering effective amount of an agonist according to claim 12 to a mammal in need of such treatment.

16. A pharmaceutical composition comprising an agonist according to claim 11, together with a pharmaceutically acceptable carrier.

17. A pharmaceutical composition comprising an antagonist according to claim 12, together with a pharmaceutically acceptable carrier.